## IN THE CLAIMS:

- 1. (currently amended) An integrated process for extracting and purifying tocotrienols/tocopherols, carotenoids and sterols and production of fatty acid esters from oils, comprising the steps of:
  - a. <u>Ttran-esterification</u> of oil containing tocotrienols/tocopherols, carotenes, sterols, fatty acids, mono-, di- and triglycerides, for a period of time at specific temperature in the presence of a monohydric alcohol, and base or acid to form an ester-rich layer and a glycerol-rich layer;
  - b. Separating the ester-rich layer from the glycerol-rich layer as obtained in (a) by gravitational settling, decantation or separation by centrifugal forces;
  - c. Wwashing and drying the ester-rich layer obtained in (b) under conditions sufficient to remove all impurities and base or acid without destroying the tocotrienols, tocopherols and carotenoids in the ester-rich layer;
  - d. <u>Ss</u>tep-wise molecular distillation or any other distillation of the resultant dried ester-rich layer as obtained in (c) to yield a concentrated mixture of tocotrienols/tocopherols, carotenoids and sterols at specific temperature and pressure;
  - e. Ffurther trans-esterification of the mixture obtained in (d) containing concentrated tocotrienols/tocopherols, carotenes, sterols, and fatty acids, mono-, di- tri glycerides, for a period of time at specific temperature in the presence of a monohydric alcohol, and base or acid to convert glycerides in the oil to form an ester-super-rich layer and a glycerol-rich layer; and
  - f. Rrepeating the above trans-esterification reactions and step-wise molecular distillations to achieve the desired concentration of tocotrienols/tocopherols, carotenoids, and sterols.

- 2. (currently amended) The process according to <u>Gclaim 1</u>, wherein the concentrated carotenoids are treated with a lower alkyl alcohol under conditions sufficient to form carotenoids miscelles without destroying the carotenoids, thereby forming a carotenoid-rich layer.
- (currently amended) The carotenoid-rich layer according to Gclaim 2, wherein the said carotenoid-rich layer is subjected to an evaporation or distillation process to distill out the lower alkyl alcohol to form a concentrated carotenoid extract.
- 4. (currently amended) The process according to Gclaim 1, wherein the concentrated tocotrienols/tocopherols/sterols mixture is treated with a low monohydric alcohol for a period of time at specific temperature to crystallize out the sterols and mono-, di-, and tri-glycerides from the mixture.
- 5. (currently amended) The concentrated tocotrienols/tocopherols/sterols mixture according to <u>Cclaim 4</u>, wherein the said mixture is subjected to a solid-liquid filtration to yield a rich tocotrienols/tocopherols filtrate and sterols cake.
- 6. (currently amended) The tocotrienols/tocopherols filtrate according to Gelaim 5, wherein the said filtrate is subjected to an evaporation or distillation process to distill out the lower alkyl alcohol to form a concentrated tocotrienols/tocopherols extract.
- 7. (currently amended) The sterols cake according to Galaim 5, wherein the sterols cake is treated with appropriate solvents for a period of time at specific temperature to concentrate the sterols.

- 8. (currently amended) The concentrated tocotrienols/tocopherols extract obtained from the crystallization process as elaimed in Eclaim 4, wherein the said tocotrienols/tocopherols concentrate is treated with appropriate solvents and absorbents, bleaching earth or activated carbon for a period of time at specific temperature to reduce the colour, and to obtain a lighter coloured tocotrienols/tocopherols concentrate.
- 9. (currently amended) The process according to Cclaim 1, wherein the oil used in the transesterification is selected from the group consisting of but not limited to, crude palm oil, crude palm olein, red palm oil, red palm olein, vegetable oil and or any other suitable edible oil.
- 10. (currently amended) The process according to <u>Gclaim 1</u>, wherein glycerides in the oil are converted to fatty acid alkyl esters and glycerol, and to form an ester-rich layer and a glycerol-rich layer, by contacting the oil with an esterification solution comprising lower alkyl alcohol and a base or acid.
- 11. (currently amended) The process according to <u>Gclaim 1</u>, wherein the ratio of oil to the esterification solution is in the range between 0.5 10 part of oil to 1 part esterification solution.
- 12. (currently amended) The process according to Cclaim 1, wherein the base used in the esterification solution is selected from a group consisting emprising of but not limited to sodium methoxide, potassium methoxide, sodium hydroxide, potassium hydroxide, and of any other suitable base.
- 13. (currently amended) The process according to Cclaim 1 or 12, wherein the ratio of base to lower alkyl alcohol in the esterification solution is in the range between 0.005 to 5 part of base to 1 part of lower alkyl alcohol.

- 14. (currently amended) The process according to Gclaim 1, wherein the acid used is selected from a group consisting comprising of but not limited to, hydrochloric acid, phosphoric acid, citric acid and or any other suitable acid.
- 15. (currently amended) The process according to Cclaim 1 or 14, wherein the ratio of acid to lower alkyl alcohol in the esterification solution is in the range between 0.005 to 5 part of acid to 1 part of lower alkyl alcohol.
- 16. (currently amended) The process according to Gclaim 1, wherein the lower alkyl alcohol used is selected from the group consisting comprising of but not limited to methanol, ethanol, butanol, propanol and or any other suitable lower alkyl alcohol.
- 17. (currently amended) The process according to <u>Gclaim 1</u>, wherein the trans-esterification is carried out at <u>a</u> temperature ranging from 5°C to 90°C with <u>a</u> time period ranging from 0.50 hour to 16 hours.
- 18. (currently amended) The process according to Gclaim 1, wherein the trans-esterification mixture is agitated at a speed of between 10 rpm to 500 rpm.
- 19. (currently amended) The process according to <u>Gclaim 1</u>, wherein the alkyl esters produced comprise methyl, ethyl, and isopropyl or butyl esters of the fatty acids, depending on the type of lower alkyl alcohol used.
- 20. (currently amended) The process according to Gclaim 1, wherein the ester-rich layer or ester-super-rich layer is separated from the glycerol-rich layer by conventional gravitational settling or centrifugal forces.

- 21. (currently amended) The process according to <u>Gclaim 1</u>, wherein the ester-rich layer or ester-super-rich layer is washed with either hot or cold water via direct contact with the hot water or through a counter-current hot water column at a temperature ranging between 30 to 90°C.
- 22. (currently amended) The process according to <u>Gclaim 1</u>, wherein the ester-rich layer or ester-super-rich layer is washed with hot water <u>untill</u> a pH of 6 to 8 is reached.
- 23. (currently amended) The process according to <u>Gclaim 1</u>, wherein the washed ester-rich layer or ester-super-rich layer is subjected to vacuum evaporation or wiped film evaporator or short path distillation to achieve a moisture content of between 0.001% to 0.20%.
- 24. (currently amended) The process according to <u>Gclaim 1</u>, wherein the dried ester-rich layer or ester-super-rich layer is subjected to a multi-stage molecular distillation at <u>a</u> temperature ranging from of 50°C to 300°C and at <u>a</u> vacuum of 0.00001 to 1.0 mbar.
- 25. (currently amended) The process according to Gelaim 1, wherein the mixture obtained in 1(d) comprises a concentrated mixture of tocotrienols/tocopherols, carotenoids and sterols at a concentration of between 0.1-10%, 0.1-10% and 0.1-10% respectively.
- 26. (currently amended) The process according to Gclaim 1, wherein the multi-stage molecular distillation of the dried ester-super-rich layer will produce tocotrienols/tocopherols/sterols extract as the distillate and carotenoids extract as the residue.

- 27. (currently amended) The process according to Galaim 1 or 26, wherein the content of tocotrienols/tocopherols/sterols in the distillate is 5% to 30% total tocotrienols/tocopherols and 5%-50% total sterols and carotenoids content in the residue is between 5% 30%.
- 28. (currently amended) The process according to Galaim 2 or 3, wherein the lower alkyl alcohol used in alcoholic washing is selected from the group consisting of but not limited to, methanol, or ethanol, or propanol, or butanol, or isopropyl alcohol and or any combination of these alkyl alcohols.
- 29. (currently amended) The process according to Gelaim 2 or 3, wherein the washing and agitation time ranges from half an hour to 30 hours and the temperature rangesing from between 5°C to 90°C.
- 30. (currently amended) The process according to Cclaim 2 or 3, wherein the concentrated carotenoids extract has a concentration of between 20%-50% total carotenoids.
- 31. (currently amended) The process according to Gelaim 2 or 3, wherein the concentrated carotenoids extract consists of alpha-carotene and beta-carotene as the major carotenoids and other carotenoids such as gamma-carotene, lycopene, phytoene and phytofluene at lower concentration.
- 32. (currently amended) The process according to any one of Cclaims 4 to 6, wherein the lower alkyl alcohol used in the crystallization of tocotrienols/tocopherols/sterols mixture is selected from the group consisting of but not limited to methanol, or ethanol, or propanol, or but and or any combination of these alkyl alcohols.

- 33. (currently amended) The process according to any one of Colaims 4 to 6, wherein the crystallization temperature ranges from 60°C to 0°C for a period ranging from 3 hours to 10 days.
- 34. (currently amended) The process according to any one of Colaims 4 to 6, wherein the evaporation temperature ranges from 10°C to 90°C.
- 35. (currently amended) The process according to any one of Cclaims 4 to 6, wherein the resulting tocols concentrate has a total concentration of tocotrienols and tocopherols ranging from between 20% to 90%.
- 36. (currently amended) The process according to any one of Cclaims 4 to 6, wherein the resulting tocols concentrate comprises all eight forms of vitamin E, namely, alpha-tocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol and alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol and delta-tocotrienol.
- 37. (currently amended) The process according to any one of Cclaims 4 to 6, wherein the resulting tocols concentrate may also contain other compounds such as squalene, sterols, carotenoids and CoQ10 with typical concentration ranging between 0.5 % 20%, 0.5% 20%, 0.05% 10% and 0.001 % 2% respectively.
- 38. (currently amended) The process according to Gelaim 5 or 7, wherein the solvent used in the purification of sterols is selected from the group consisting of but-not-limited to hexane, heptane, iso-octane, acetone, or ethyl acetate and or any combination of these solvents, in the ratio ranging from between 1:1-10:1.
- 39. (currently amended) The process according to Gelaim 5 or 7, wherein the crystallization of sterols is carried out at a temperature ranging from

- 30°C to 10°C for 12 to 72 hours and the resulting filtered and dried sterols have a total phytosterols content ranging from 70% to 90%.
- 40. (currently amended) The process according to Gelaim 5 or 7, wherein the temperature range of the purification of sterols is between 10°C to 80°C and the time period ranges ing from 1 to 10 hours.
- 41. (currently amended) The process according to Gclaim 8, wherein the temperature range of the decolourization process is between 10°C to 90°C and the time period ranges ing from 1 to 24 hours per batch.
- 42. (currently amended) The process according to Gclaim 8, wherein the mixture is agitated in the range from 10 rpm to 1000 rpm.
- 43. (currently amended) The process according to Calaim 8, wherein the mixture after reaction is filtered with filter press or vacuum filtration or centrifugation or simple settling and the resulting filtrate is evaporated at temperature ranging from 10°C to 90°C and at a vacuum of between 1 mbar to 0.0001 mbar.
- 44. (currently amended) The process according to Galaim 8, wherein the final decolourized tocols concentrate has a colour range of between 1R to 20R when measured with a 5-1/2 inch cell of the Lovibond Tintometer.
- 45. (currently amended) Tocotrienols, tocopherols, carotenoids and sterols produced from oils according to the process as elaimed in claim 1. any one of the preceding claims.
- 46. (new) The process according to claim 12, wherein the ratio of base to lower alkyl alcohol in the esterification solution is in the range between 0.005 to 5 part of base to 1 part of lower alkyl alcohol.

- 47. (new) The process according to claim 14, wherein the ratio of acid to lower alkyl alcohol in the esterification solution is in the range between 0.005 to 5 part of acid to 1 part of lower alkyl alcohol.
- 48. (new) The process according to claim 26, wherein the content of tocotrienols/tocopherols/sterols in the distillate is 5% to 30% total tocotrienols/tocopherols and 5% -50% total sterols and carotenoids content in the residue is between 5% 30%.
- 49. (new) The process according to claim 3, wherein the lower alkyl alcohol used in alcoholic washing is selected from the group consisting of but not limited to, methanol, or ethanol, or propanol, or butanol, or isopropyl alcohol and or any combination of these alkyl alcohols.
- 50. (new) The process according to claim 3, wherein the washing and agitation time ranges from half an hour to 30 hours and the temperature ranges ing-from between 5°C to 90°C.
- 51. (new) The process according to claim 3, wherein the concentrated carotenoids extract has a concentration of between 20%-50% total carotenoids.
- 52. (new) The process according to claim 3, wherein the concentrated carotenoids extract consists of alpha-carotene and beta-carotene as the major carotenoids and other carotenoids such as gamma-carotene, lycopene, phytoene and phytofluene at lower concentration.
- 53. (new) The process according to claim 5, wherein the lower alkyl alcohol used in the crystallization of tocotrienols/tocopherols/sterols mixture is

selected from the group consisting of but not limited to methanol, or ethanol, or propanol, or butanol and or any combination of these alkyl alcohols.

- 54. (new) The process according to claim 6, wherein the lower alkyl alcohol used in the crystallization of tocotrienols/tocopherols/sterols mixture is selected from the group consisting of but not limited to methanol, or ethanol, or propanol, or butanol and or any combination of these alkyl alcohols.
- 55. (new) The process according to claim 5, wherein the crystallization temperature ranges from 60°C to 0°C for a <u>time</u> period ranging from 3 hours to 10 days.
- 56. (new) The process according to claim 6, wherein the crystallization temperature ranges from 60°C to 0°C for a time period ranging from 3 hours to 10 days.
- 57. (new) The process according to claim 5, wherein the evaporation temperature ranges from 10°C to 90°C.
- 58. (new) The process according to claim 6, wherein the evaporation temperature ranges from 10°C to 90°C.
- 59. (new) The process according to claim 5, wherein the resulting tocols concentrate has a total concentration of tocotrienols and tocopherols ranging from between 20% to 90%.
- 60. (new) The process according to claim 6, wherein the resulting tocols concentrate has a total concentration of tocotrienols and tocopherols ranging from between 20% to 90%.

- 61. (new) The process according to claim 5, wherein the resulting tocols concentrate comprises all eight forms of vitamin E, namely, alphatocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol and alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol and delta-tocotrienol.
- 62. (new) The process according to claim 6, wherein the resulting tocols concentrate comprise all eight forms of vitamin E, namely, alphatocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol and alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol and delta-tocotrienol.
- 63. (new) The process according to claim 5, wherein the resulting tocols concentrate may also contain other compounds such as squalene, sterols, carotenoids and CoQ10 with typical concentration ranging between 0.5 % 20%, 0.5% 20%, 0.05% 10% and 0.001 % 2% respectively.
- 64. (new) The process according to claim 6, wherein the resulting tocols concentrate may also contain other compounds such as squalene, sterols, carotenoids and CoQ10 with typical concentration ranging between 0.5 % 20%, 0.5% 20%, 0.05% 10% and 0.001 % 2% respectively.
- 65. (new) The process according to claim 7, wherein the solvent used in the purification of sterols is selected from the group consisting of but not limited to hexane, heptane, iso-octane, acetone, or ethyl acetate and or any combination of these solvents, in the ratio ranging from between 1:1-10:1.

- 66. (new) The process according to claim 7, wherein the crystallization of sterols is carried out at a temperature ranging from 30°C to 10°C for 12 to 72 hours and the resulting filtered and dried sterols have a total phytosterols content ranging from 70% to 90%.
- 67. (new) The process according to claim 7, wherein the temperature range of the purification of sterols is between 10°C to 80°C and the period ranging from 1 to 10 hours.

## Conclusion

The Applicant respectfully requests entry of this preliminary amendment. Please contact the Applicant's below-signed attorneys with any questions.

Respectfully submitted,

David G. Oberdick, Esquire

Reg. No. 39,527

Christian M. Best, Esquire

Reg. No. 49,082

Customer No. 42161

Meyer, Unkovic & Scott, LLP

1300 Oliver Building

Pittsburgh, PA 15222

Tel: (412) 456-2588

Attorneys for Applicant